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The Histopathology of Rat Lung Following Exposure to Zinc Oxide/Hexachloroethane Smoke or Instillation with Zinc Chloride Followed by Treatment with 70% Oxygen

by Roger F. R. Brown,* Timothy C. Marrs,* Paul Rice,* and Lisa C. Masek†

The effects of inhaled zinc oxide/hexachloroethane smoke (11,580 mg•min/m³) and intratracheally instilled zinc chloride (2.5 mg/kg body weight) have been studied in rat lung. The effects of subsequent treatment with 70% oxygen have been studied after both procedures.

Both the inhalation of the smoke and instillation of zinc chloride produced similar effects that included pulmonary edema, alveolitis and, at a later stage, some fibrosis. After zinc chloride instillation, the pathological changes largely spared the periphery of the lung, while following smoke inhalation they were more diffuse. Subsequent oxygen administration had little effect on the development or progression of the pathological changes.

Introduction

Screening smokes have a variety of uses, both civilian and military. One method of generating a white screening smoke is by igniting a pyrotechnic mixture containing zinc oxide and a chloride donor such as hexachloroethane. The reaction produces a number of compounds, including zinc chloride, zinc oxychloride, and hydrogen chloride. Phosgene and carbon tetrachloride may also be produced, although the concentration in the smoke cloud is likely to be low (*1*).

Evidence accumulated over the years indicates that, in certain circumstances, these smokes can produce morbidity. This is exemplified by numerous case reports (*2–6*). Although most cases have recovered, the course of the case reported by Matarese and Matthews (*6*) was prolonged, and in another case the patient died (*3*).

Zinc oxide-hexachloroethane smoke has been the subject of a number of animal studies. Thus, Cullumbine (*7*) studied the acute effects of the smoke in mice and guinea pigs and found that the latter died rapidly after exposure and that the Lct_{50} in mice was 11,800 mg•min/m³. The no-effect dose in mice, as far as macroscopic or microscopic lung damage was concerned, was 2000 mg•min/m³. The main organ-specific effects were tracheobronchitis, pulmonary congestion, and edema.

Ardran (*8*) carried out a radiological investigation of dogs exposed to the smoke and showed there was hemoconcentration together with the radiological appearances of pulmonary edema. Ardran states that the edema took longer to clear than that produced by phosgene.

Marrs et al. (*9*) exposed rabbits and rats to single doses of two different zinc oxide/hexachloroethane smokes and found inflammation and, in some cases, necrosis of the laryngeal and tracheal mucosa. Pulmonary edema and pneumonitis were seen in the dead animals.

Karlsson et al. (*10*) compared zinc oxide-hexachloroethane smoke with one generated from titanium and not only found the zinc smoke to be the more toxic of the two, but also confirmed previous findings of pulmonary edema. In the rat, the Lct_{50} for a 10-min exposure was 2000 mg/m³. These workers also studied the time course of the development of the histological changes and showed a variety of later effects including atelectasis, the presence of inflammatory cells, aggregates of macrophages, and bronchiolo-alveolar hyperplasia.

The effect of oxygen on chemically induced lung damage is complex. Oxygen can be toxic (*11*) yet, alternatively, it is widely used to combat the hypoxemia of

*Pathology Section, Procurement Executive, Ministry of Defence, Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, SP4 OJQ, UK.

†Department of Biochemistry, University College, Cardiff, Wales, CF 1XL, UK.

Address reprint requests to R. F. R. Brown, Pathology Section, Procurement Executive, Ministry of Defence, Chemical Defence Establishment, Porton Down, Salisbury, Wilshire, SP4 OJQ, UK.

pulmonary edema. When used either in conjunction with or after the administration of paraquat (*12,13*) or butylated hydroxytoluene (*14*), oxygen aggravates the toxicity. Furthermore, oleic acid-induced lung damage results in increased tolerance to high concentrations of oxygen (*15*).

The purpose of the present series of experiments was to determine the effects on the lung of both ignited zinc oxide/hexachloroethane smoke when inhaled, and a major component of the smoke, zinc chloride ($ZnCl_2$) that was administered intratracheally. Since oxygen is recommended in the treatment of the pulmonary edema caused by zinc oxide/hexachloroethane smokes (*3,16*), the effects of treatment with 70% oxygen after the inhalation of the smoke or instillation of $ZnCl_2$ was also investigated.

Materials and Methods

Animals

Male Porton Wistar-derived rats (200–250 g) were obtained from the Animal Breeding Unit, Chemical Defence Establishment, Porton Down, UK. They were fed *ad libitum* and maintained at 20°C with an ambient humidity of 40 to 60%.

Materials

Zinc oxide/hexachloroethane powder consisting of 46% hexachloroethane, 40% zinc oxide, and 14% calcium silicide was manufactured by Chemical Defence Establishment (Porton Down, UK). Commercial 100% oxygen was obtained from BOC plc (Southampton, UK), and all other chemicals, from BDH plc, (Poole, UK). For intratracheal instillation the $ZnCl_2$ was dry heat sterilized at 140°C for 2 hr, then dissolved in sterile distilled water to give a stock solution of 1.25 mg/kg. This was diluted before using with sterile 0.15 M NaCl.

Smoke Exposure

The animals were exposed, in groups of five, housed in wire cages, to zinc oxide/hexachloroethane smoke in a 10 m^3 chamber for a period of 60 min. The smoke was generated by igniting 6.5 g of powder and maintained by periodically burning the powder during the exposure. Gravimetric measurements of the smoke were made by sampling onto 55-mm diameter, Whatman glass fiber filter papers (Grade B) at a flow rate of 5.6 L/min. Weighing the solid matter allowed calculations of the particulate solid concentration in the smoke. The zinc content was not measured since numerous previous experiments have showed that the zinc content was approximately 20% (*17*).

Oxygen Exposure

A total of 40 animals were exposed to zinc oxide/hexachloroethane smoke, and immediately following the exposure they were divided into two groups of 20. One group was not treated further and was kept under ambient oxygen concentrations in the animal house. The other 20 were further divided into groups of 5, housed in wire cages, and placed into a 1-m^3 chamber. Oxygen (100%) was continuously bled into the chamber at a rate of 10 to 12 L/min, while the chamber was evacuated at a rate of 18 L/min by means of a Austin diaphragm pump. The oxygen concentration was monitored using a Taylor Servomex Sybron Corporation (Birmingham, UK) oxygen analyzer (type OA 250) coupled to a pen recorder. The animals were exposed continuously to 70 ± 3% oxygen for 3 days, except for short periods for daily feeding and watering.

The numbers of animals per group and times of post mortem after their exposure to zinc oxide/hexachloroethane smoke or zinc oxide/hexachloroethane smoke and oxygen are shown in Table 1. Two control groups of five animals that received no exposures were sacrificed at 3 days and 28 days.

Intratracheal Instillation of Zinc Chloride

Groups of five animals were lightly anesthetized with halothane vapor (Fluothane) and then were intratracheally instilled by using the method of Richards et al. (*18*) with a sterile solution of $ZnCl_2$ at a dose of 2.5 mg/kg body wt. A control group of five animals was instilled with the solvent only. A total of 40 rats were instilled with $ZnCl_2$, and immediately following instillation they were divided into two groups of 20; one of these groups, after further division into subgroups of five animals, was exposed to oxygen as described above. The remainder, again in subgroups of five animals, were kept under ambient oxygen conditions in the animal house. The times of post mortem examination, after instillation of $ZnCl_2$ with and without subsequent exposure to 70% oxygen, is shown in Table 2. The animals were observed daily and weighed at least three times a week.

Table 1. The experimental protocol for zinc oxide/hexachloroethane smoke exposure[a].

Time of sacrifice	Control	Control +70% oxygen	Zn/HCE[b] smoke	Zn/HCE smoke +70% oxygen
3 days	5	5	8	5
14 days			8	5
28 days	5	5	9	5

[a]Ct (concentration × time in min) 11,580 mg•min/m^3.
[b]HCE, hexachloroethane.

Table 2. The experimental protocol for the instillation of zinc chloride, 2.5 mg/kg body weight.

Time of sacrifice	Control	Control +70% oxygen	Zn/HCE[b] smoke	Zn/HCE smoke +70% oxygen
3 days	5	5	5	5
14 days	5	5	5	5
28 days	5	5	5	5
35 days	5	5	5	5

Pathology

After sacrifice with a lethal dose IP of Sagatal (60 mg/mL sodium pentabarbitone), the lungs were inflated *in situ* with 10% neutral-buffered formalin, and samples for histological examination were taken by median transverse section from the upper and lower lobes of the right lung and from the left lung. After routine processing and embedding in paraffin wax, 4-μm sections were cut and stained with hematoxylin and eosin, Masson's Trichrome, van Gieson's stain for collagen, Martius Scarlet Blue for fibrin, and silver stain for reticulin.

Results

Smoke Inhalation

The mean concentration for the zinc oxide/hexachloroethane exposure was 193 g/L giving, a Ct = 11,580 mg•min/m^3. All animals showed some respiratory distress during their exposure to zinc oxide/hexachloroethane smoke, and on removal from the chamber, they were very subdued. By 1 hr after exposure, however, no adverse signs were detected. A total of 11 of the animals that were exposed to zinc oxide/hexachloroethane smoke died during the first 3 days following their exposure. No histopathological examination was carried out on these animals, but gross pathology indicated the presence of severe pulmonary edema.

Instillation of Zinc Chloride

Within 3 hr of instillation of $ZnCl_2$ all animals were very subdued and showed some respiratory distress. Two of the animals in the oxygen-treated group died between 24 and 48 hr after instillation; again, macroscopic examination of the lung indicated the presence of gross pulmonary edema.

Pathology

The main changes observed after all treatments were edema, destructive alveolitis, and macrophage infiltration, followed by the development of fibrosis (Figs. 1–4).

Three days after zinc oxide/hexachloroethane smoke exposure, or at the end of the oxygen treatment period, the most consistent finding was pulmonary edema and some isolated areas of macrophage infiltration. There was no difference between the oxygen-treated and untreated groups. By 14 days after the zinc oxide/hexachloroethane smoke exposure, pulmonary edema was no longer present, but some fibrosis, mainly interstitial in distribution, was observed. Macrophage infiltration was also observed in the lungs of both groups. Some focal Type II pneumocyte hyperplasia was seen in the lungs of the oxygen-treated group. At 28 days after zinc oxide/hexachloroethane smoke exposure, both oxygen-treated and untreated groups showed more widespread fibrosis and macrophage infiltration with no difference being observed between the different treatments.

Histological changes to the lung after $ZnCl_2$ instillation were patchy and generally centrilobular in distribution; $ZnCl_2$ alone caused destructive alveolitis around the major bronchi, which was most severe at 3 days. The alveolitis appeared to be somewhat less extensive in those animals instilled with $ZnCl_2$ and then treated with oxygen. By 14 days, reparative processes and a change from predominantly macrophage to lymphocytic infiltration was seen in the areas of alveolar damage. Small aggregates of foamy macrophages were observed in the alveolar lumina. There was no evidence of fibrosis at this stage. At 28 days, early alveolar thickening with increased interstitial reticulin deposition was observed (Fig. 5). By 35 days, these changes had amounted to mature, discrete areas of parenchymal scarring (Fig. 6). At 14, 28, and 35 days the oxygen-treated group appeared similar but somewhat less severely affected (Figs. 7,8).

Histological examination of the lungs from control animals that were sacrificed at 3 and 28 days revealed a generally normal appearance with isolated instances of mild perivascular or peribronchiolar lymphocytic infiltration. The lungs of animals that had been instilled with saline and then exposed to 70% oxygen showed evidence of perivascular edema with associated pulmonary venous congestion.

Discussion

The changes observed after zinc oxide/hexachloroethane smoke are similar to those previously described (*9,10*). Thus, Marrs et al. (*9*) observed alveolitis and edema in dead animals from a study using inhaled zinc oxide/hexachloroethane smoke from two different compositions. Some of the histological findings were very florid in the earlier study, doubtless because the exposure concentration was more than 10 times that used in the present study. Fibrosis was not seen in the Marrs et al. (*9*) study, but the animals were only retained for 14 days. Karlsson et al. (*10*) in their comparative study of titanium and zinc smokes also observed pulmonary edema, Type II alveolar pneumocyte hyperplasia, and infiltration with inflammatory cells. In the same study, exposure of rats to $ZnCl_2$ aerosols produced similar pathological effects. This suggests that $ZnCl_2$ is indeed an important component of the pyrotechnically generated smoke. In the present study, there were considerable similarities between the histological appearances of the lung after inhalation of zinc oxide/hexachloroethane smoke and intratracheal administration of $ZnCl_2$, reinforcing this view. Thus, pulmonary edema was observed after both experimental procedures, as was fibrosis. There was, however, a noteworthy difference in that after instillation the periphery of the lungs was spared and the appearance of pathological changes were markedly patchy. After inhalation of the smoke the observed changes were diffuse in distribution. At

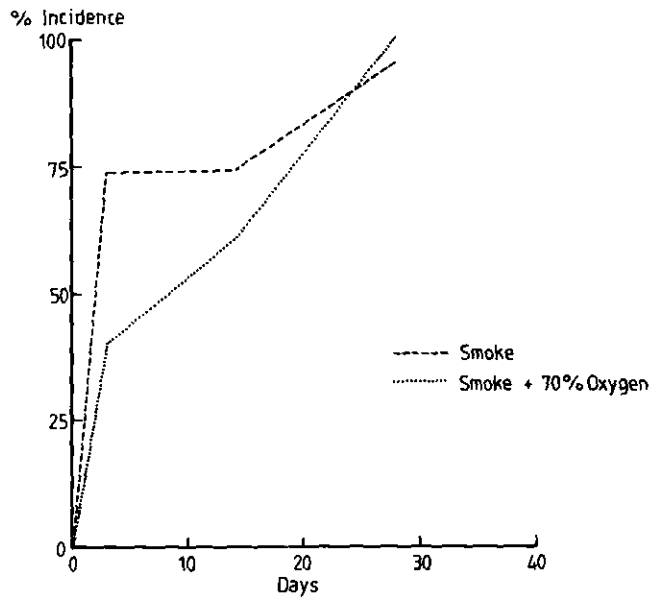

FIGURE 1. Macrophage infiltration in the lungs of rats exposed to zinc oxide/hexachloroethane smoke and oxygen at varying time intervals after the cessation of exposure. Incidence = the percentage of animals treated with zinc oxide/hexachloroethane smoke with or without subsequent oxygen therapy in which macrophage infiltration was observed. Animals were killed at intervals of up to 35 days after exposure. For full details see text.

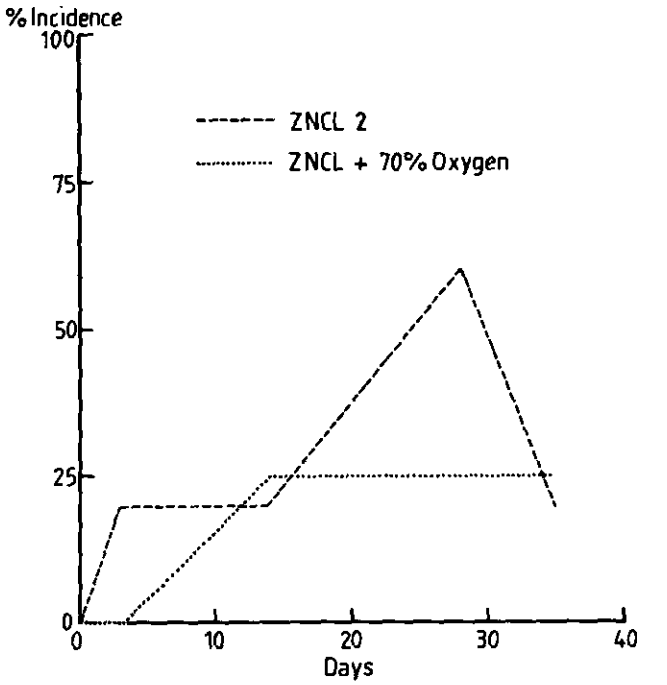

FIGURE 3. Macrophage infiltration in the lungs of rats following instillation of ZnCl and subsequent treatment with oxygen at varying time intervals after the cessation of exposure. Incidence = the percentage of animals treated with $ZnCl_2$ with or without subsequent oxygen therapy in which macrophage infiltration was observed. Animals were killed at intervals of up to 35 days after exposure. For full details see text.

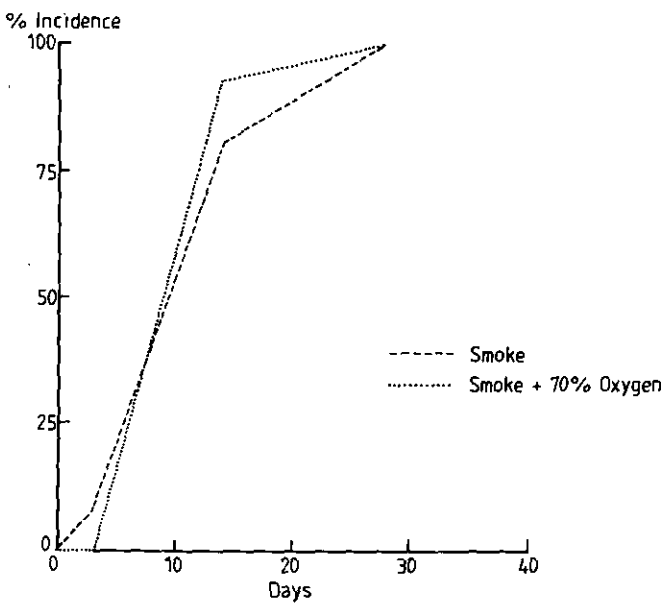

FIGURE 2. Pulmonary fibrosis in the lungs of rats exposed to zinc oxide/hexachloroethane smoke and oxygen at varying time intervals after cessation of exposure. Incidence = the percentage of animals treated with zinc oxide/hexachloroethane smoke with or without subsequent oxygen therapy in which pulmonary fibrosis was observed. Animals were killed at intervals of up to 35 days after exposure. For full details see text.

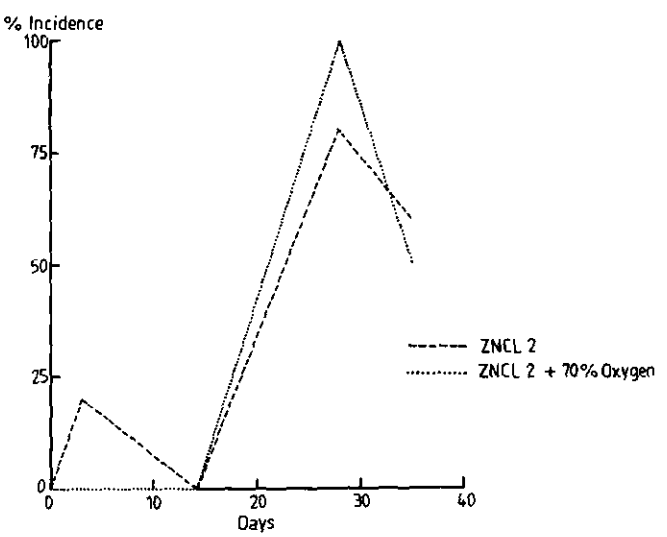

FIGURE 4. Pulmonary fibrosis in the lungs of rats following instillation with $ZnCl_2$ and subsequent treatment with oxygen at varying time intervals after the cessation of exposure. Incidence = the percentage of animals treated with $ZnCl_2$ with or without subsequent oxygen therapy in which macrophage infiltration was observed. Animals were killed at intervals of up to 35 days after exposure. For full details see text.

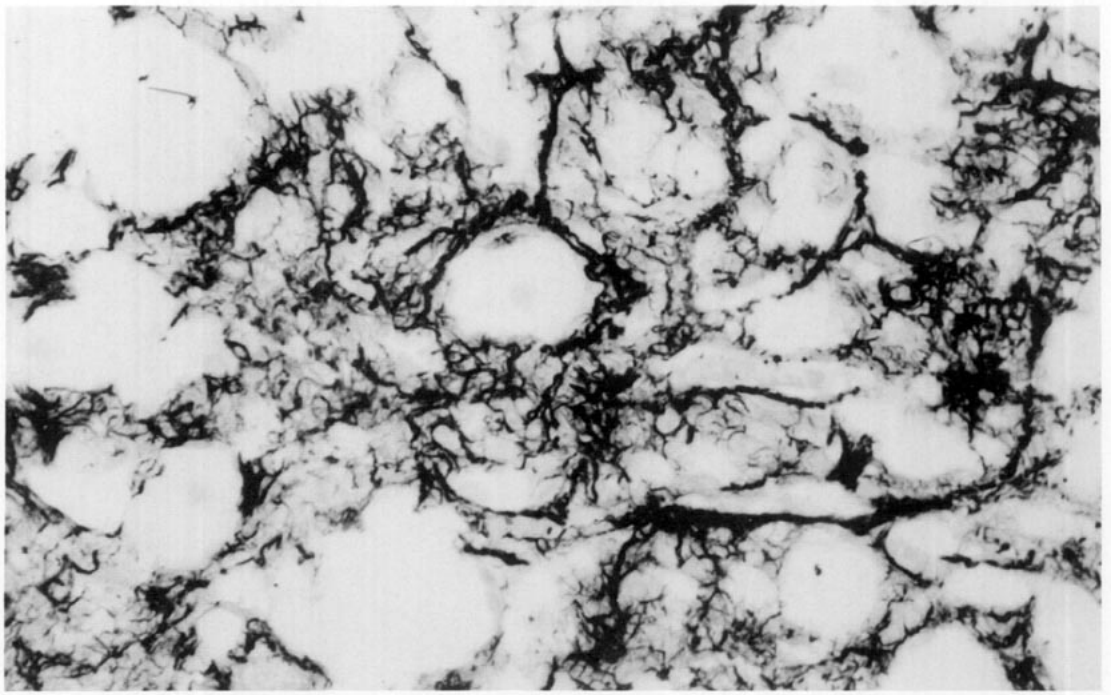

FIGURE 5. Photomicrograph of lung from animal that had been intratracheally instilled with $ZnCl_2$ (2.5 mg/kg) and killed 28 days after the instillation. Showing an area of early interstitial fibrosis. Reticulin. ×355.

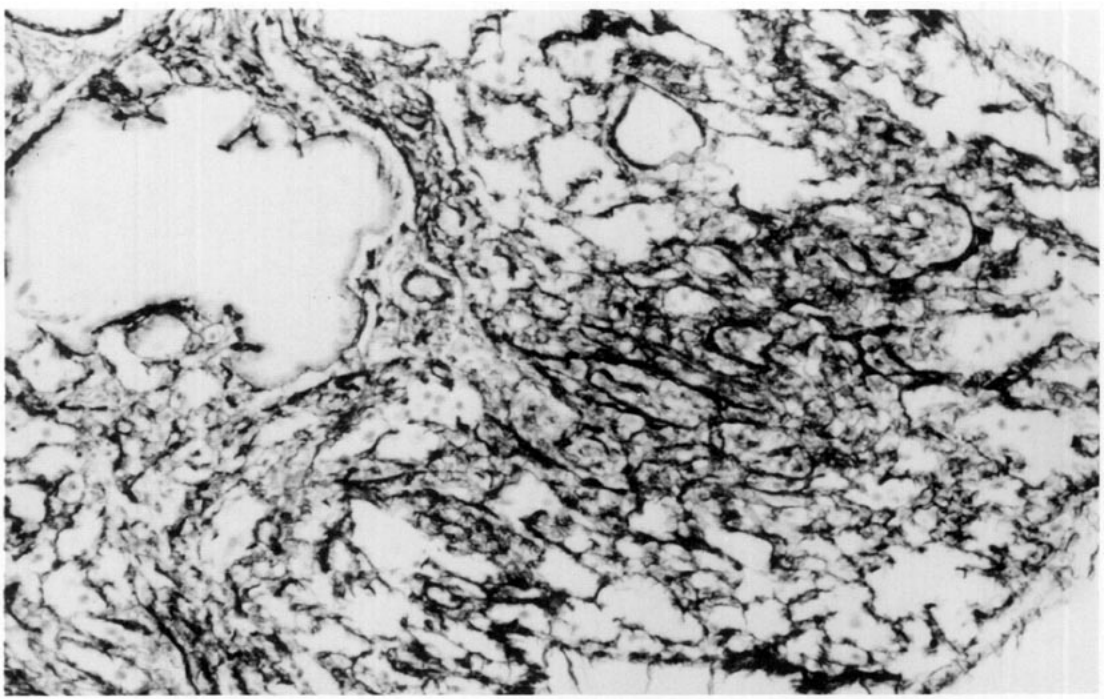

FIGURE 6. Photomicrograph of lung from animal which had been intratracheally instilled with $ZnCl_2$ (2.5 mg/kg) and killed 35 days after the instillation. Showing an area of fibrosis with small bronchiole left upper corner. Reticulin. ×221.

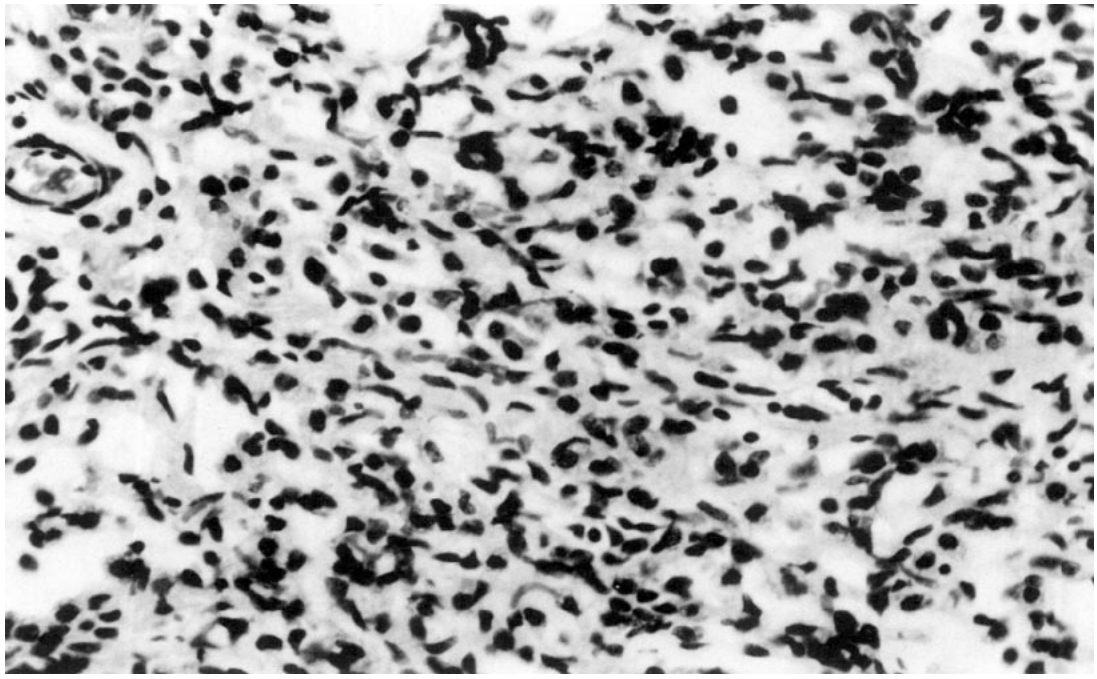

FIGURE 7. Photomicrograph of lung from animal which had been intratracheally instilled with $ZnCl_2$ (2.5 mg/kg) and killed 35 days after the instillation. Showing an area of fibrosis with grey intercellular staining of collagen. H&E, ×575.

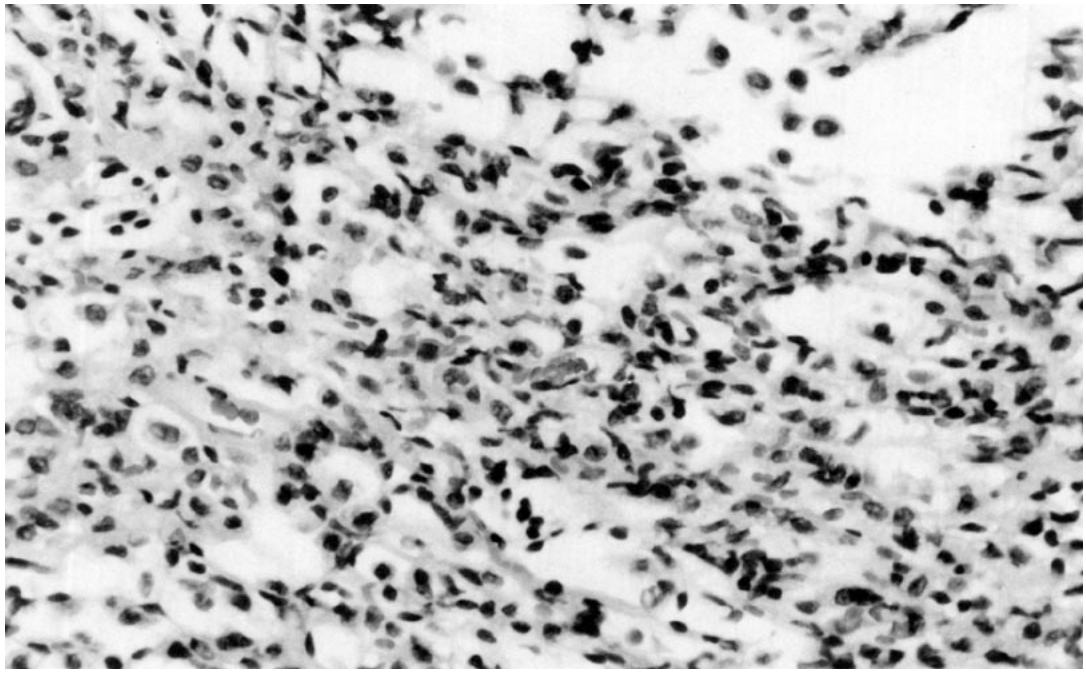

FIGURE 8. Photomicrograph of lung from animal which had been intratracheally instilled with $ZnCl_2$ (2.5 mg/kg) and killed 35 days after the instillation. Showing an area similar to Figure 3 showing increased collagen in an area of fibrosis. Masson's Trichrome, ×355.

later stages of the study, animals exposed by inhalation had diffuse fibrosis, while rats given $ZnCl_2$ by intratracheal instillation had areas of fibrous scarring. These differences are most likely explained by the fact that, intratracheally, instilled fluids tend to remain localized around the main bronchi while particulate aerosols tend to reach out further into the periphery.

The effect of additional treatment with oxygen was not marked, but the observed differences between corresponding groups suggests that both after inhalation and instillation, oxygen was mildly beneficial. This suggests that zinc oxide/hexachloroethane smoke poisoning is not likely to be aggravated by treatment with oxygen, as is the case with some other lung toxicants (*12–14*). Also, the suggested recommendation to use oxygen (*16*) is probably wise, particularly when pulmonary edema is severe.

Clearly, zinc oxide/hexachloroethane smoke produces a severe chemical alveolitis, and human exposure is likely to produce severe respiratory signs and symptoms. Even optimum treatment with steroids, both inhaled and parenterally administered, as well as penicillamine (*19*) are likely to result in a slow resolution. Nevertheless, some reassurance may be found in that in a repeated animal dose study, after cessation of exposure for 1 year, no residual fibrosis was seen in rats or mice (*17*).

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